in caldesmon and certain myosins. This novel structural motif contains a high proportion of charged E, R and K residues, which appear as alternating patches of like-charged residues throughout the sequence. The resulting salt bridge interactions between E and R/K sidechains are thought to stabilise and stiffen the straight helical structure, allowing the SAH to act as a spacer between two flanking functional domains. Here we use single molecule force spectroscopy and molecular simulation to investigate the mechanical unfolding behaviour of the ~100 residue SAH domain from myosin 10. Both methods indicate a globally non-cooperative unfolding process, with unfolding occurring below ~50 pN. Simulations suggest that the SAH domain does differ from a non-charged helix, not only in the stability of the helix but also in the unfolding characteristics under application of force. Enhanced local bonding interactions in SAH domains increases their resilience to force above the baseline level set by a non-charged helix.

1313-Pos Board B43

Interactions of Urea with the Folded and Unfolded States of Proteins

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Urea is a strong protein denaturant, yet, the mechanism of action of urea on the protein unfolding process is still largely unknown. To tackle this problem, we determined the partial molar volumes, V^o, and adiabatic compressibilities, K^o_S, of a set of model proteins to examine their interactions with urea. Specifically, we measured the partial molar volume and adiabatic compressibility of ribonuclease A, α-chymotrypsinogen A, lysozyme, and apocytochrome c in aqueous solutions of urea at concentrations between 0 and 8M. At pH 2 and pH3, ribonuclease A and α-chymotrypsinogen A, respectively, exhibit a two-state transition of unfolding over the range of urea concentrations studied. Even in 8M urea, lysozyme retains its native conformation and apocytochrome c remains unfolded at pH 7. The fact that lysozyme and apocytochrome c do not undergo any conformational transitions in the presence of urea provides an opportunity to study the interactions of urea with proteins in the native and unfolded states within the entire range of experimentally accessible urea concentrations. We analyze our resulting volumetric data within the framework of a statistical thermodynamic model in which each instance of urea interaction with a protein is viewed as a binding reaction that is accompanied by release of two water molecules. From this analysis, we calculate the association constants, k, as well as changes in volume, ΔV_O, and adiabatic compressibility, ΔK_{SO}, accompanying each urea-protein association event in an ideal solution. By comparing these parameters with similar characteristics determined for low-molecular weight analogues of proteins, we quantify the extent of cooperative effects involved in interactions of urea with any of the proteins studied in this work.

1314-Pos Board B44

Volume Changes Upon Unfolding of Globular Proteins: Computational and Experimental Studies

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Hydrostatic pressure is an important environmental variable that plays an essential role in biological adaptation. Increasing pressure, much like increasing temperature, perturbs the thermodynamic equilibrium between folded state and unfolded state. In thermodynamic terms stability is defined by Gibbs energy, *G*, that is function of both temperature and pressure and is related to the population of native, N, and unfolded, U, states aswhere K_{eq} is equilibrium constant. The pressure dependence of *G* is defined by the volume changes between the unfolded and native states. What is the origin of the changes in volume of a protein upon unfolding? The presence of internal cavities and voids in the interior of the native proteins makes the volume of the native protein larger that the volume of the unfolded state. However, the hydration of internal protein groups that become exposed to the solvent upon unfolding should also contribute to the changes in volume. Based on model compound data, the hydration in expected to contribute negatively to the changes in the *V* specific volume of a protein upon unfolding. As a result it appears that the unfolding of proteins should be accompanied by a large decrease in their volumes. However, in most cases, only small decreases, or even small increases, in the partial specific volume of proteins upon unfolding are observed. To reconcile these observations, we have analyzed volume changes due to the internal cavities and volume using structural ensembles of proteins generated using various computational models including an all-atom explicit solvent molecular dynamics simulations. We show that previous calculations significantly overestimated the volume changes due to the cavities and voids.

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1315-Pos Board B45

Conformational Flexibility and Structure in High-Pressure Excited States of Apomyoglobin Revealed by SDSL-EPR

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Proteins exist in an ensemble of conformations at equilibrium, and while higher energy (''excited'') states may play important functional roles, under normal conditions they are too sparsely populated to detect. The application of high hydrostatic pressure may be used to populate excited states, but characterization of the high-pressure conformational ensemble is complicated by the presence of multiple conformations exchanging on the μs-ms time scale. The method of site-directed spin labeling (SDSL) in combination with electron paramagnetic resonance (EPR) is ideally suited to explore such excited states. The intrinsic timescale of EPR (ns) is fast compared to that of conformational exchange, so the EPR spectrum captures a snapshot of conformational equilibrium frozen in time and can reveal the existence of multiple conformational substates. For each resolved substate, the spectral lineshape encodes information on the local backbone dynamics and tertiary fold. In the present work, a set of spin-labeled mutants of both holo- and apomyoglobin were studied in the pressure range 0 - 2 kbar. Many EPR spectra in the well-ordered holomyoglobin native state are essentially pressure independent, demonstrating that the internal motion of the spin label side chain is pressure insensitive. Thus, pressure-dependent spectral changes observed in more flexible systems can be directly interpreted in terms of protein compressibility rather than side chain effects. Pressurization of apomyoglobin up to 2 kbar populates a low-lying excited state that has been designated a ''high-pressure molten globule,'' although the work presented here shows it to be distinct from the conventional low pH molten globule that is an intermediate along the folding pathway. Interestingly, the EPR spectra of apomyoglobin at 2 kbar suggest the existence of a conformation with native-like dynamics within the ensemble of conformations that comprise the high-pressure molten globule.

1316-Pos Board B46

Single Protein Complexes Isomerization and Conformational Dynamics Using Trapped Ion Mobility Spectrometry: From MS to Seconds

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In the present work, examples of protein and peptide complexes conformational dynamics, from the solvent state distribution to the gas-phase ''de-solvated'' state distribution, are characterized for traditionally considered ''unstructured'' complexes. Conformational motifs and isomerization/conformational dynamics are identified and isomerization kinetics in the ms to few seconds timescale are measured for single molecules using a trapped ion mobility spectrometer - mass spectrometer (TIMS-MS). Theoretical calculations are used to simulate the experimental ''TIMS box'' single molecule -neutral bath gas phase dynamics and candidate structures are proposed for each conformational state. It is found that, side chain and backbone structural changes are the main motifs governing the conformational inter-conversion processes in the ms-s time scale. Examples will be shown for the case of folded/unfolded protein complexes and DNA-binding proteins.

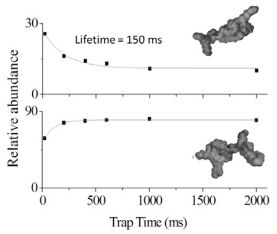

Figure. Example of an isomerization/conformational dynamic pathway for the the $[M+2H]^{+2}$ truncated form of the HMGA2 DNA-binding protein (ATHP) containing the third DNA binding motif.

1317-Pos Board B47

Minima and Barriers on the Pressure-Temperature Free Energy Landscape of Phosphoglycerate Kinase

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Proteins can exhibit strikingly different free energy landscapes depending on which thermodynamic variable is manipulated to study folding. We have investigated the relationship between temperature- and pressure-induced denaturation of yeast phosphoglycerate kinase (PGK). Thermodynamically, the pressure-temperature phase diagram of PGK seems standard: with a slight deviation from two-state behavior at low temperatures, a predicted elliptical shape of stability emerges. Kinetically, the behavior is much more complex: although a temperature perturbation of the energy